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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Receptor Induced by Lymphocyte Activation in Inflammatory Response

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ABSTRACT

A receptor (ILA) isolated and substantially purified from lymphoid cells activated with proinflammatory agents is disclosed along with its nucleotide and amino acid sequences. Also disclosed are diagnostic and therapeutic methods of utilizing the ILA nucleotide and polypeptide sequences. ILA is also induced in primary chondrocytes by IL-1β and cytokines that cause cartilage degradation.

A RECEPTOR INDUCED BY LYMPHOCYTE ACTIVATION IN INFLAMMATORY RESPONSE

This invention was made with Government support under Grant Nos. CA51406 and AR39799, awarded by the National Institutes of Health. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

1. *Field of the Invention*

This invention relates to controlled regulation of host defense responses by cytokines and their receptors. More specifically, this invention relates to identification and characterization of a human receptor induced in lymphoid and other cell lineages by proinflammatory stimuli.

2. *Description of Related Art*

Inflammation can be viewed as one aspect of the host defense response that confines and repairs injury, and immunity can be viewed as another aspect of the host defense response that specifically neutralizes invading microbes and confers specific resistance to future infection by the same invader. In reality these aspects are inseparable, and most cells and molecules that defend the body are involved in both inflammation and immunity.

In the first phase of the response to injury white blood cells known as polymorphonuclear leukocytes, or granulocytes, leave the flowing blood and adhere to endothelial cells, lining blood vessels. The endothelial cells spread apart somewhat and enable the granulocytes to pass into the injured tissue, where they ingest and destroy any microbes that may have entered the wound.

Next macrophages join in and replace the granulocytes as the predominant cell type at the injury, swallowing damaged cells and bacteria, particularly those coated with antibody. At the same time T lymphocytes proliferate and arouse other defensive cells, including B lymphocytes, which divide, differentiate and secrete antibodies in quantity. As the infection is controlled, connective-tissue cells called fibroblasts and other cells begin to repair damaged tissue.

Among the most recent important advances in immunology have been the purification, molecular cloning and functional characterization of a wide array of regulatory proteins through which cells can communicate with one another during operation of the host defense response. These molecules are variously known as lymphokines or cytokines. A number of these, such as interleukin-2 (IL-2), one of the proteins that regulate the growth of T cells and B cells, IL-3, IL-4, IL-5, IL-6, granulocyte-macrophage colony stimulating factor (GM-CSF), gamma interferon, and tumor necrosis factor, an immune system-derived protein that can inhibit the growth of a variety of cell types, including tumor cells, have been purified and the genes encoding them have been cloned and expressed. It has been shown that these molecules act on their target cells by binding to high-affinity receptors expressed on those cells. The structure of the receptors and the means through which growth factor - receptor interactions lead to biologic functions are now issues of central importance in the study of immune responses. Use of the pure proteins and of antibodies to them as tools to study the function of lymphokines and related regulatory and effector molecules in the immune system has disclosed that individual lymphokines have a multiplicity of functions and the same function is often exerted by more than one lymphokine.

The biological activity of cytokines as mediated of host defense responses is controlled through regulated expression of the cytokines as well as the receptors. Cloning of cytokine receptor genes has resulted in the identification of several receptor families that contain structurally related receptors (A. Miyajima, *et al.*, *Annu. Rev. Immunol.*, 10:295, 1992). The NGF/TNF receptor family, which is active in host defense response, is characterized by the presence of 3-6 cysteine-rich motifs of approximately 40 amino acids in the extracellular domain (S. Mallet, *et al.*, *Immunol. Today*, 12:220, 1991). Members of this family include the low affinity NGFR (D. Johnson, *et al.*, *Cell*, 47:545,1986) TNFR-I (Schall, *et al.*, *Cell*, 61:361, 1990; Loetscher, *et al.*, *Cell*, 61:351, 1990) TNFR-II (Smith, *et al.*, *Science*, 248:1019, 1990) CD40 (Stamenkovic, *et al.*, *EMBO J.*, 8:1403, 1989), CD30 (Durkop, *et al.*, *Cell*, 68:421, 1992), CD27 (Camerini, *et al.*, *J. Immunol.*, 147:3165, 1991), Fas-APO-1 (Itoh, *et al.*, *Cell*, 66:233, 1991; Oehm, *et al.*, *J. Biol. Chem.*, 267:10709, 1992), OX-40 (Mallett, *et al.*, *EMBO J*, 9:1063, 1990), 4-1BB (Kwon, *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:1963, 1989). These receptors recognize soluble or cell-surface bound ligands and mediate diverse cellular responses. TNF and NGF regulate cell proliferation and secretory functions in different cell types (Mallet, *supra*; Beutler, *et al.*, *Annu. Rev. Immunol.*, 7:625, 1989). Stimulation of cells through the TNFR and the Fas/APO-1 antigen can induce apoptosis (Yonehara, *et al.*, *J. Exp. Med.*, 169:1747, 1989; Trauth, *et al.*, *Science*, 245:301, 1989). In contrast, antibody to the B cell antigen CD40 in combination with antibody to cell surface immunoglobulin can prevent apoptosis (Liu, *et al.*, *Nature*, 342:929, 1989) and promote long term maintenance of B cells (Banchereau, *et al.*, *Science*, 251:70, 1991). Stimulation of CD27, which is expressed on most peripheral blood T cells and mature thymocytes, and of the rat T cell subset antigen, OX-40, by specific antibodies increases lymphocyte proliferation (van Lier, *et al.*, *J. Immunol.*, 139:1589,

1987; Bigler, *et al.*, *J. Immunol.*, 141:21, 1988). Most recently 4-1BB, a murine T lymphocyte specific antigen, has been suggested to recognize extracellular matrix proteins (Chalupay, *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:10360, 1992) and to function as an accessory signaling molecule during T cell activation (Polluk, *et al.*, *J. Immunol.*, 150:771, 1993).

The members of the NGF/TNFR family differ in their tissue distribution. The murine 4-1BB and the rat OX-40 are most restricted and only expressed on certain T cell subsets. CD40 appears to be specific for B lineage cells, while CD30 can be found on activated normal T and B cells as well as on Hodgkin's malignant human lymphoid cell lines. CD27 has been detected on T and B lymphocytes, mature thymocytes and some chronic B lymphocytic leukemias. The Fas/APO-1 antigen has been found on lymphoid and myeloid cells as well as on diploid fibroblasts (Yonehara, *et al.*, *supra*). In contrast to these receptors that appear to be restricted to T and B lymphocytes is the broad tissue distribution of the NGFR and the two TNFRs. Although NGF has originally been identified on the basis of its ability to promote survival of sensory and sympathetic neurons, the NGFR is now known to also be expressed on lymphocytes and to regulate immune function (Otten, *Proc. Natl. Acad. Sci. USA.*, 86:10059, 1989). TNFα and TNFβ, the ligands for the TNFR, also regulate function of cells in a variety of different tissues.

Tissue distribution of cytokine receptors and the production of soluble receptors are additional mechanisms that determine cytokine function. Receptors for regulatory factors such as cytokines, growth factors, or neuropeptides can be expressed at relatively similar levels during different activation states of a cell, or expression levels can depend on stimulation with specific agents. Through conservation of three to six cysteine-rich extracellular

domains, usually 41-44 amino acids in length, the members of the nerve growth factor/tumor necrosis factor ("NGF/TNF") receptor family appear to share a common structure that is suitable for ligand binding. Signal transduction through these receptors has only partially been characterized and diverse mechanisms appear to be used.

For instance, the NGF receptor itself displays only low affinity for NGF. On the other hand, high affinity binding of NGF is obtained upon association with the trk oncogene product, which by itself binds NGF and the related factor NT3, but brain-derived neurotrophic factor ("BDNF"), does not even though all three of these factors increase neuron survival (Klein, *et al.*, *Cell*, 65:189, 1991; Ragsdale, *et al.*, *Nature*, 350:660, 1991). Another putative mechanism involves a threonine residue conserved in the intracellular domains of CD40, Fas/APO-1, the NGF receptor ("NGFR"). It has been determined that this residue is phosphorylated in response to antibody binding to CD40 (Inui, *et al.*, in Leukocyte Typing IV) and may represent a conserved mechanism of signal transduction.

Several members of the NGF/TNF family are found in soluble forms. Soluble forms of the low affinity NGFR, the two TNFRs and CD27 have been described (Hintzen, *et al.*, *J. Neuroimmunol.*, 35:211-217, 1991). These proteins are capable of binding their respective ligands and have the effect of inhibiting the biological activity of the ligand. Soluble TNFRs appear to be generated by proteolytic cleavage of the membrane associated forms, since for each of these receptors only a single mRNA species has been detected. This is in contrast to the soluble IL-4 (Mosley, *et al.*, *Cell*, 59:335-348, 1989) and IL-7 receptors (Goodwin, *et al.*, *Cell*, 60:941-951, 1990) which are both derived from alternatively spliced mRNAs.

In addition, several members of the NGF/TNF family appear to be characterized by activation-dependent expression. Dependence of expression levels upon specific activation, generally by cytokines, has been demonstrated for CD40 by Stamenkovic, *et al.*, *supra*, for CD27 by van Lier, *et al.*, *supra*, for 4-1BB by Kwon, *et al.*, *Cell. Immunol.*, 121:414-422, 1989) and for Fas/APO-1 by Trauth, *et al.*, *supra*.

Inflammation and immunity cause a range of uncomfortable symptoms and disability. If the defensive response is too vigorous or goes on too long, as it does when an infection is chronic, or when the body treats self-generated molecules as foreign, it can do permanent harm. One important example of autoimmune disease is rheumatoid arthritis, in which chronic inflammation can produce debilitating effects. In articular cartilage, liberation of lymphokines during the inflammatory response leads to accumulation of macrophages within the synovium and to synthesis of immunoglobulin and rheumatoid factor. Ultimately, the immune complexes in articular cartilage attract PMNs, which damage cartilage by releasing proteases and oxygen radicals.

To date no satisfactory treatments for chronic inflammatory arthritis exist. Gold shots or a potable gold composition, such as auranofin, invite drastic side effects in many patients. Intermittent corticosteroids reduce inflammation, but can lead to degenerative arthritis. Immunosuppressive agents such as alkylating agents (chlorambucil, cyclosphamide), purine analogs (mercaptopurines, azathioprine) and antimetabolites (methotrexate) have all been used, but can increase incidence of neoplasm and infection. *Harrison's Principles of Internal Medicine*, 11th Ed., editors E. Braunwald, *et al.*, McGraw-Hill Book Company, New York, pp. 1423-1427, 1987.

New and further information concerning the molecular biology involved in immunological response to inflammatory stimuli, such as arthritis, awaits discovery of additional receptors associated with host defense responses. The present study identifies a new member of the human NGF/TNF family. This receptor gene can be induced in lymphoid and different non-lymphoid cell types by proinflammatory stimuli such as IL-1β, suggesting that it may be involved with adaption during host defense responses. By cloning the complementary DNA sequence encoding the human receptor induced in lymphoid and other cell lineages by proinflammatory stimuli, the primary structure of this receptor can be established and its role in the inflammatory response can be further investigated. Such studies could potentially lead to the design of new anti-inflammatory agents.

SUMMARY OF THE INVENTION

A 400 bp polymerase chain reaction (PCR) product was identified during analysis of receptor gene expression in human T lymphocytes. This product was not detected in resting cells but was induced by lymphocyte activation and is known as "ILA". The sequence of a full length 1.4 kb cDNA was obtained by screening a library prepared with RNA from HTLV-1 transformed human T cells activated with PHA (phytohemaglutin) and PMA (phorbol 12-myristate 13-acetate). Sequence analysis identified this cDNA as a new member of the NGF/TNF receptor family. The isolation of this particular cDNA was not anticipated nor predictable since the primers used in the polymerase chain reaction corresponded to sequences in a distinct family of receptors that are characterized by 7 transmembrane regions and associate with G-proteins. This new cDNA had the highest degree of homology with the murine T cell specific receptor 4-1BB. The gene encodes 3 cysteine-rich motifs in the extracellular domain that are characteristic of this receptor family, a transmembrane region, and a short N-terminal cytoplasmic portion with potential phosphorylation sites. ILA is a receptor for an as-yet-unidentified factor.

Analysis of poly-A$^+$ RNA from activated human peripheral blood T lymphocytes by Northern blotting identified transcripts at 4.4, 4.0 and 1.8 kb. These mRNAs were not detected in unstimulated cells. An RNA-PCR assay was used to study tissue distribution and regulation of ILA expression. The gene was induced in T lymphocytes by PHA, PMA and antibody to CD3; in B lymphocytes by PMA and antibodies to cell surface immunoglobulin, and anti-μ; and in blood monocytes by IL-1β, LPS and PMA. In T lymphocytes, ILA mRNA was detectable 1.5 hours after stimulation, reached maximal levels at 8 hours and declined to background levels by 48 hours. Induction was primarily due

to increased transcription as actinomycin D reduced ILA mRNA levels in activated lymphocytes to 50% within 30 minutes, demonstrating a relatively short half life of this mRNA.

Analysis of non-lymphoid cells showed that ILA mRNA was not detectable in resting cells. However, in contrast to the lymphoid-specific expression of the murine 4-1BB gene, ILA was detected in non-lymphoid cells including epithelial, hepatoma and connective tissue cells after stimulation with IL-1β.

These results identify ILA as a new member of the human NGF/TNF receptor family and show that expression of this receptor gene is activation-dependent. In addition, ILA is found in a broad tissue distribution. It can be induced in T lymphocytes as well as in B lymphocytes, monocytes, and diverse non-lymphoid cell types.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows a cDNA nucleotide sequence and deduced amino acid sequence for a receptor induced by lymphocyte activation (ILA). The signal peptide and the transmembrane domain are underlined. The potential polyadenylation site is indicated in bold letters. Potential N-linked glycosylation sites are marked by asterisk. Potential phosphorylation sites were found at position 242 for protein kinase C (PKC) and at positions 234 and 235 for caseine kinase II (CKII).

FIGURE 2 shows a hydropathicity profile for the predicted ILA amino acid sequence. Values were determined according to the method of Kyte and Doolittle. Positive values indicate hydrophilic regions, and negative values stand for hydrophobic regions. The amino acid positions are presented on the horizontal axis.

FIGURE 3 shows consensus sequence alignment of the deduced amino acid sequence of ILA with murine 4-1BB. Identical amino acids are indicated by vertical lines. Amino acids with high, low, and no similarity are indicated by colons, points, and blanks, respectively.

FIGURE 4 shows expression of ILA mRNA in peripheral blood mononuclear cells (PBMC). RNA was isolated from unstimulated cells (lanes 1 and 2) or from cultures that had been activated with PHA (1μg/ml) and PMA (1ng/ml) for 11 hours (lanes 3 and 4). Total RNA (15 μg/lanes 1 and 3) or poly A^+ RNA (1μg/lanes 2 and 4) was analyzed for the presence of ILA transcripts. Figure 4A shows analysis by Northern blotting. As shown in Figure 4B to document the amount of RNA loaded, the filters were subsequently analyzed for cyclophilin mRNA. The difference in intensity of the signals in panel B resulted from use of total (lanes 1 and 3) rather than poly A^+ (lanes 2 and 4) RNA.

FIGURE 5 shows time course of ILA expression. PBMC were stimulated with PHA (1μg/ml) and PMA (1ng/ml). RNA was isolated at the indicated time points 1) 4 hours; 2) 8 hours; 3) 18 hours; 4) 30 hours; 5) 48 hours.

FIGURE 6 shows time course of ILA expression in response to T cell activation by anti-CD3. PBMC were stimulated with anti-CD3 antibody. RNA was isolated at the indicated time points and analyzed by RT-PCR. To document equal amount of template the samples were also analyzed for G3PDH mRNA.

FIGURE 7 shows ILA mRNA stability in PHA/PMA stimulated and actinomycin D treated PBMC. Known amounts of an ILA deletion clone were co-amplified in the same reactions. FIGURE 7A shows PCR products run on a 1.3% agarose gel and stained by ethidium bromide. FIGURE 7B is a plot of data obtained from scanning a negative of the gel shown in Figure 7A. Control: squares; 15 min actD: diamonds; 30 min actD: circles.

FIGURE 8 shows ILA expression in primary and in EBV transformed B-lymphocytes. Primary (lanes 2-4) and EBV transformed (lanes 5 and 6) B-lymphocytes were stimulated for 6 h and analyzed for ILA mRNA by RT-PCR. Lane 1: negative PCR control; Lane 2: unstimulated; Lane 3: PMA (10 ng/ml), Lane 4: anti-μ (12.5 μg/ml); Lane P: PhiX 174, HaeIII markers; Lane 5: unstimulated; Lane 6: PMA (10 ng/ml). To document equal amounts of template, the samples were also analyzed for G3PDH mRNA.

FIGURE 9 shows ILA induction in monocytes by PMA and IL-1β. Peripheral blood monocytes were stimulated with PMA and IL-1β at the indicated concentrations. In Figure 9A, RNA was isolated after 4 h and analyzed for ILA mRNA by Northern blotting. Since the load of RNA differed considerably in this experiment a rehybridization of the blot with G3PDH cDNA is shown in Figure 9B: Lane 1 control; Lane 2: PMA (100 ng/ml); Lane 3: PMA (1 ng/ml), Lane 4: PMA (10 ng/ml; Lane 5: PMA (50 ng/ml); Lane 6: IL-1β 10 ng/ml. Figure 9C shows a plot of the results after densitometry and normalization of the ILA signals on the basis of G3PDH signals.

FIGURE 10 shows ILA induction in non-lymphoid cells by IL-1β. HEp-2 (epithelioid), Hep-G2 (hepatoma) cells or human articular chondroycles were stimulated with IL-1β (10ng/ml) for 6 hours. The presence of ILA mRNA was determined by PCR. To document that equal amounts of template were used, the samples were also analyzed for G3PDH mRNA. Lane P: PhiX, Hae III (Stratagene); Lane 1: Negative PCR control; Lane 2: Hep-G2, control; Lane 3: Hep-G2, IL-β; Lane 4: Chondrocytes, control; Lane 5. Chondrocytes, IL-β; Lane 6: Negative PCR control; Lane 7: HEp-2, control; Lane 8: HEp-2, IL-β; Lane 9: A549 (lung carcinoma).

FIGURE 11 shows ILA is not inducible in neuronal cell lines. HS683 cells were stimulated for 8 h and analyzed by RT-PCR for ILA. Lane 0: no template; Lane 1: unstimulated control; Lane 2: IL-1β (10 ng/ml); Lane 3: IFNγ (500 U/ml); Lane 4: PMA (5 ng/ml) + A23187 (1 mM); Lane 5: positive control; M: marker = PhiX174, HaeIII.

A DETAILED DESCRIPTION OF THE INVENTION

This invention provides a novel peptide, known herein as "ILA" and identified as inducible in lymphoid and other cell lineages by proinflammatory stimuli. A 1.4 kb full-length 1419 bp cDNA encoding ILA (Seq. I.D. No. 1) (shown in Figure 1) was isolated from a library constructed from activated human T cell leukemia virus type 1 transformed human T lymphocytes. The cDNA is deposited with GenBank under Accession No. L12964.

As shown in Figure 1, ILA is a 255 amino acid with a molecular mass of approximately 28 kD (SEQ. I.D. No. 2). Hydropathicity analysis of the amino acid sequence, shown in Figure 2, predicts a putative signal peptide (amino acids 1 to 17) and a 27 amino acid hydrophobic region that is a potential transmembrane domain (amino acids 187 to 213) flanked by charged residues. Following the 17 amino acid leader peptide, ILA is characterized by an extracellular domain of 169 amino acids, a transmembrane region of 27 amino acids, and a short intracellular domain of 42 amino acids. Based on these features, ILA can be characterized as a type I transmembrane protein.

Two potential N-glycosylation sites at positions 138 and 149 are based on the presence of a NXS/T motif (with X being any amino acid except D or P). The serine at position 242 is a potential site for phosphorylation by protein kinase C. The two consecutive threonines at positions 234 and 235 are potential sites for phosphorylation by casein kinase II (Kueuzel, *et al.*, *J. Biol. Chem.*, 161:9136, 1993).

The extracellular part of ILA contains 3 cysteine-rich repeats characteristic of members of the NGF/TNFR superfamily. The second of these is composed of only 32 instead of the usual 41-44 amino acids. The cysteine-rich extracellular domain of ILA, which is comparable to those founding members of the NGF/TNF family, is substantially similar to the extracellular domain of murine 4-1BB. It is believed that through conservation of the cysteine-rich extracellular domains the members of the NGF/TNF receptor family share a common structure for ligand binding.

Comparison of ILA with sequences in the National Biology Research Foundation (NBRF) database showed that it has the highest degree of homology with the murine cDNA sequence 4-1BB (Figure 3) which is expressed by specific T-lymphocyte subsets (Kwon, *supra*). The deduced amino acid sequences of ILA and 4-1BB display 73.6% similarity and 59.6% identity as based on sequence comparison using NIH software.

Amino acids 241 to 244 of ILA (CSCRFD) contain a sequence similar to the sequence (CSCRCP, amino acids 239 to 244) in 4-1BB that has been identified as a possible ligand binding site associated with activation of the tyrosine kinase $p56^{lck}$. This sequence in ILA, which differs by only one amino acid from that of 4-1BB, has been confirmed in two independently isolated cDNAS and suggests that this potential signalling mechanism is conserved between 4-1BB and ILA.

Three different isoforms of ILA mRNA were detected in activated PBMC. The most abundant form was at 4.4 kb and this was the only species of ILA readily detectable in total cellular RNA. Poly A^+ RNA was necessary for clear detection of the forms at 4.0 and 1.8 kb. The smallest form at 1.8 kb contains

the full length coding region while the larger size of the two additional species is probably due to extended 3' untranslated regions. The difference between the 1.4 kb cDNA and the smallest transcript of 1.8 kb is probably due to priming of oligo dT on an internal A rich region, since six independent cDNA clones of 1.4 kb were isolated.

ILA mRNA was not detected in PBMC from more than 20 healthy donors using a sensitive PCR assay. However, ILA was rapidly inducible by PHA, PMA and anti-CD3. Transcripts were detected as early as 90 minutes after stimulation with PHA/PMA. Immediate early genes, such as c-jun and c-fos, are inducible within 15 minutes according to Nakamura, *et al.* (*Cell Growth and Differentiation*, 2:267, 1991). ILA thus appears to be an early activation gene.

A competitive PCR was performed to determine the stability of the ILA mRNA. At 15 and 30 minutes after addition of a known transcriptional inhibitor, actinomycin D, to activated PBMC, ILA mRNA decreased to 78% and 47% of control levels, respectively. Based on these values, the calculated half life of ILA mRNA is approximately 30 minutes. This characterizes ILA as a highly unstable mRNA. By comparison, in activated monocytes the half lives of the c-jun and c-fos transcripts, which are among the most unstable mRNAS, was determined to be 27 and 25 minutes, respectively (Nakamura, *et al.*, *supra*).

Unlike 4-1BB, which is expressed only in T lymphocytes, ILA can be induced in all major cellular subsets of the immune system including B lymphocytes, monocytes and macrophages and at early as well as late stages of their differentiation. Its expression in all normal lymphoid cell types tested was activation-dependent.

ILA was induced in blood monocytes in response to their major activators including LPS, IL-1β and PMA. In addition, it appeared that monocyte adherence is also a stimulus for the induction of this mRNA. ILA was not detected in freshly isolated blood monocytes but was induced upon adherence of the cells to polystyrene tissue culture dishes. U937 was used as a cell line representing an early state in monocyte differentiation, and *in vivo* derived macrophages were used as representing terminally differentiated cells. In both cell types ILA was inducible in response to PMA or IL-1β.

B lymphocytes were isolated from peripheral blood of normal donors, purified, and ILA expression was induced by antibodies to cell surface immunoglobulin or PMA. Analysis of B cell lines showed that EBV transformation is associated with the constitutive expression of ILA mRNA. Collectively, these results show that, within the immune system, ILA can be much more broadly expressed than has been described for 4-1BB. The broad distribution of ILA indicates that ILA potentially serves as a molecule in mediating communication among all cell types of the immune system as well as functioning as the recipient on T lymphocytes for signals derived from other antigen presenting cells such as B lymphocytes or monocytes.

The results from the *in vitro* studies suggested that ILA mRNA can be expressed by different cell types in response to stimulation. These observations did not provide evidence for a role of this receptor in normal or pathogenic immune responses *in vivo*. To address this question, we analyzed RNA that was extracted from human tonsils, as sites where normal immune responses occur and found ILA mRNA expressed in all samples. To test the notion that ILA may be involved with aberrant immune responses that lead to

disease, we examined mononuclear cells from rheumatoid arthritis patients. All synovial fluid samples contained high levels of ILA mRNA.

Collectively, these results suggest that ILA participates in normal immune responses and is involved with the pathogenesis of arthritis. ILA appears to participate in immune responses physiologically and in disease as indicated by its presence in lymphocytes from tonsil and sites of autoimmune reactions.

In nonlymphoid cells the proinflammatory cytokine IL-1β is a strong inducer of ILA. In contrast, TGFβ, which is a qualitatively distinct cytokine and antagonizes some of the proinflammatory effects of IL-1, did not induce ILA mRNA expression. IL-1 is involved in coordination of cell function in response to trauma or infection. Based on the activation-dependence and IL-1 inducibility of ILA, it is likely that ILA is a receptor that is involved with the cellular adaptation during host defense response to injury in diverse tissues.

Of further significance to the role of ILA in host defense responses is its expression in a broad spectrum of non-lymphoid cells. The analysis of tissue distribution performed in the present study clearly shows that ILA is inducible in human articular chondrocytes, one example of a normal mesenchymal cell type, as well as in hepatoma and epithelial tumor cell lines, such as lung carcinoma. It is of interest that ILA was not inducible in any of the different cell lines established from brain tumors, possibly indicating that ILA is not a product of neuronal cell types.

The other members of the NGF/TNFR family differ in their tissue distribution. The rat OX-40 is most restricted and only expressed by certain T cell subsets.

CD40 appears to be specific for B lineage cells; while CD30 can be found on activated normal T and B cells as well as on Hodgkin's lymphoma cells. Fas/APO-1 is also expressed on T and B lymphocytes and on malignant human lymphoid cell lines. CD27 has been detected on T and B lymphocytes, mature thymocytes and some chronic B lymphocytic leukemias. The Fas/APO-1 antigen had been found on lymphoid and myeloid cells as well as on diploid fibroblasts (Yonehara, *et al., supra*). In contrast to these receptors that appear to be restricted to T and B lymphocytes is the broad tissue distribution of the NGFR and the two TNFR. The broad tissue distribution of ILA provides proof that it is a member of the NGF/TNF receptor family known for its wide tissue distribution.

The discovery of ILA makes possible further study of the inflammatory host defense to discover the natural ligands for the ILA receptor and the binding proteins that regulate production of ILA. In addition, the discovery of ILA makes possible diagnosis and therapy of ILA mediated pathologies. For example, ILA can be used to produce polyclonal or monoclonal antibody preparations and other ILA agents that are specifically reactive with ILA or the DNA encoding ILA.

The term "ILA agents" as used herein is meant to include such antibodies as well as ILA ligands, including the to-be-discovered native ligand for the ILA receptor, inhibitors of the ILA receptor, and fragments of these molecules.

The term "substantially pure" or substantially purified" is meant to denote that the protein is substantially free of other compounds with which it is normally associated. The term is meant to describe a protein which is homogeneous

by one or more purity or homogeneity characteristics used by those of ordinary skill in the art.

The term "fragment" is meant to include both synthetic and naturally-occurring amino acid or sugar sequences derivable from the naturally-occurring sequence.

The term "ILA-mediated pathology" denotes disorders in which the ILA receptor contributes to the disease condition either directly or indirectly and includes cells of non-lymphoid origin which have the ILA receptor on their surface. Examples of disorders which are mediated by the ILA receptor includes atherosclerosis, autoimmune disease, transplant rejection, pathogenic host defense responses to microorganisms, and malignancy. Malignancies of particular relevance are tumors such as lung carcinoma.

DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization procedures which are well known in the art These include, but are not limited to : 1) hybridization of probes to genomic or cDNA libraries to detect shared nucleotide sequences; 2) antibody screening of expression libraries to detect shared structural features and 3) synthesis by the polymerase chain reaction (PCR).

Hybridization procedures are useful for the screening of recombinant clones by using labeled mixed synthetic oligonucleotide probes where each probe is potentially the complete complement of a specific DNA sequence in the hybridization sample which includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA.

Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al., Nucleic Acid Research*, 9:879, 1981).

An ILA-containing cDNA library can be screened by injecting the various mRNA derived from cDNAS into oocytes, allowing sufficient time for expression of the cDNA gene products to occur, and testing for the presence of the desired cDNA expression product, for example, by using antibody specific for ILA or epitopes located thereon, or by using probes for conserved regions of the sequence and a tissue expression pattern characteristic of ILA. Alternatively, a cDNA library can be screened indirectly for ILA proteins having at least one epitope using antibodies specific for ILA or fragments thereof. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of ILA cDNA.

The term "antibody" as used in this invention is meant to include intact molecules as well as fragments thereof, such as, for example, Fab and $F(ab')_2$, single chain, antibodies and any genetically engineered binding ligand that contains or is derived from the antigen binding region of an antibody

Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the

sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA, denatured double-stranded DNA or mRNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al., supra*).

The development of specific DNA sequences encoding ILA, or fragments thereof, can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA. Of these three methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, *et al., Nucl. Acid Res.*, 11:2325, 1383).

A cDNA expression library, such as lambda gt11, can be screened indirectly for ILA or fragments thereof having at least one epitope, using antibodies specific for ILA. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of ILA cDNA.

DNA sequences encoding ILA can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such

progeny are included when the term "host cell" is used. Methods of stable transfer, in other words when the foreign DNA is continuously maintained in the host, are known in the art.

In the present invention, the cDNA sequences encoding ILA may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the ILA genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg, *et al.*, *Gene*, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein I, or polyhedrin promoters and enhancer).

Polynucleotide sequences encoding ILA peptides can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention.

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the $CaCl_2$ method by procedures well known in the art. Alternatively, $MgCl_2$ or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell or by electroporation.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the ILA proteins of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein (*Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

Isolation and purification of microbially expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

RNA from any cell can be copied into double stranded DNA and cloned, resulting in the production of a cDNA library specific to the cell type. In order for such clones to be optimally useful, full-length RNA must be used as the

starting material. Since most ribonucleases are very stable and require no co-factors to function, cells are lysed in a chemical environment that denatures ribonuclease, such as a nonionic detergent or guanidinium solution. The RNA is then density fractionated from other cellular macromolecules, usually by centrifugation, and quantitated spectrophotometrically (*Current Protocols in Molecular Biology*, Ed. by F.M. Ausubel, Current Protocols, Vol. 1, §4.2, 1993).

Most messenger RNAs contain a poly A tail, while structural RNAs do not. Poly A selection therefore enriches for messenger RNA. Poly A$^+$ RNA can be separated from the remainder of total RNA, which is largely tRNA and rRNA. The total RNA can be denatured to expose the Poly A tails. Poly A-containing RNA is then passed through a column having bound oligo(dT) with the remainder of the RNA washing through. The poly A$^+$ RNA is eluted by removing salt from the solution, thus destabilizing the dT:RNA hybrid. The column separation step can be repeated to remove contaminating poly A$^-$ RNA.

Procedures such as S1 nuclear analysis using M13 as template and ribosome protection can be used to do fine-structure mapping of any RNA to determine the level, structure, size, and synthesis rate of RNA. For analyzing in detail RNA structure and amount, S1 analysis, ribonuclease protection, primer extension and Northern blots can be used. Both S1 analysis (*Current Protocols*, §4.6) and ribonuclease protection (*Current Protocols, supra*, §4.7) use a single-stranded probe that is complementary to the sequence of the measured RNA. These protocols can be used to determine both the endpoint and the amount of a specific RNA. The S1 technique uses an end-labeled single-stranded DNA probe for unambiguous determination of the 5' end of a message. Ribonuclease protection, on the other hand, increases sensitivity by utilizing a body-labeled RNA probe.

The technique of primer extension (*Current Protocols, supra* §4.8) employs a labeled oligomer of defined sequence that is extended to the end of any homologous RNA by the enzyme reverse transcriptase. In this technique RNA mapping proceeds across discontinuities, such as splice sites. Alternatively, by Northern blot hybridization (*Current Protocols, supra* §5.0). RNA is separated on an agarose gel and transferred to a filter, usually nitrocellulose. The size and amount of any specific RNA is determined by hybridizing a labeled specific probe to the nitrocellulose filter. This allows determination of the size of the entire message and also is very sensitive to message level. And finally, the nuclear runoff technique (*Current Protocols, supra* §4.10) is used to determine how the transcription rates of genes vary in response to the growth state of a cell. Consequently this technique can be used to discover whether a cell generates ILA mRNA in response to activation by proinflammatory agents such as lymphokines.

The invention includes polyclonal and monoclonal antibodies immunoreactive with ILA polypeptides or immunogenic fragments thereof. If desired, polyclonal antibodies can be further purified, for example, by binding to and elution from a matrix to which ILA polypeptide is bound. Those of skill in the art will know of various other techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies. Antibody which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler, *et al., Nature*, 256:495, 1975). The term antibody or, immunoglobulin as used in this invention includes intact molecules as well as genetically engineered antibody constructs such as bifunctional antibodies.

CDR grafted antibodies, and the like, as well as fragments thereof, such as Fab and $F(ab')_2$, which are capable of binding an epitopic determinant on ILA.

A preferred method for the identification and isolation of an antibody binding domain that exhibits binding with ILA peptides is the bacteriophage λ vector system. This vector system has been used to express a combinatorial library of Fab fragments from the mouse antibody repertoire in *Escherichia coli* (Huse, *et al.*, *Science*, 246:1275-1281, 1989) and from the human antibody repertoire (Mullinax, *et al.*, *Proc. Natl. Acad. Sci.*, 87:8095-8099, 1990). As described therein, receptors (Fab molecules) exhibiting binding for a preselected ligand were identified and isolated from these antibody expression libraries. This methodology can also be applied to hybridoma cell lines expressing monoclonal antibodies with binding for a preselected ligand. Hybridomas which secrete a desired monoclonal antibody can be produced in various ways using techniques well understood by those having ordinary skill in the art and will not be repeated here. Details of these techniques are described in such references as *Monoclonal Antibodies-Hybridomas: A New Dimension in Biological Analysis*, Edited by Roger H. Kennett, *et al.*, Plenum Press, 1980; and, U.S. Patent No. 4,172,124.

The ILA polynucleotide in the form of an antisense polynucleotide is useful in treating disease states associated with formation of ILA receptors i.e., rheumatoid arthritis, particularly in articular cartilage, by preventing expression of the protein that is associated with the host defense response to proinflammatory agents. Essentially, any disorder which is etiologically linked to expression of ILA receptor could be considered susceptible to treatment with a reagent of the invention which modulates ILA expression. The term "modulate" envisions the suppression of expression of ILA when it is

over-expressed, or augmentation of ILA expression when it is under-expressed or when the ILA expressed is a mutant form of the polypeptide. When inflammatory host defense response is associated with ILA overexpression, such suppressive reagents as antisense ILA polynucleotide sequence or ILA binding antibody can be introduced to a cell. Alternatively, when an host defense disorder is associated with underexpression or expression of a mutant ILA polypeptide, a sense polynucleotide sequence (the DNA coding strand) or ILA polypeptide can be introduced into the cell.

The invention provides a method for detecting a cell expressing ILA or a host defense disorder associated with expression of ILA comprising contacting a cell or component in a cell suspected of expressing ILA or of having an ILA associated disorder, with a reagent which binds to the component. For instance, the reagent binds to substantially purified ILA, or binds to activated lymphoid or other cells induced to express ILA, but does not bind to resting cells, which do not express the component. The cell component can be nucleic acid, such as DNA or RNA, or protein. When the component is nucleic acid, the reagent is a nucleic acid probe or PCR primer. When the cell component is protein, the reagent is an antibody probe. The probes are detectably labeled, for example, with a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator or an enzyme. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody or probe, or will be able to ascertain such, using routine experimentation.

For purposes of the invention, an antibody or nucleic acid probe specific for ILA or fragments thereof may be used to detect the presence of ILA polypeptide (using antibody) or polynucleotide (using nucleic acid probe) in

biological fluids or tissues. Any specimen containing a detectable amount of antigen can be used. A preferred sample in this invention is T or B cells. More praferably a sample is tissue of articular cartilage origin, specifically primary chondrocytes. Preferably the subject is human.

Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, pyridoxal, and fluorescein, which can react with specific anti-hapten antibodies.

The method for detecting a cell expressing ILA or a host defense disorder associated with expression of ILA, described above, can be utilized for prescreening for detection of potentially dangerous inflammatory response prior to a subject's manifestation of typical clinical features of disorders associated therewith such as systemic inflammatory defense syndrome, rheumatoid arthritis and the like. Additionally, the method for detecting ILA polypeptide in cells is useful for prescreening to detect risk of inflammatory disorder by identifying cells activated by proinflammatory agents and hence expressing ILA at levels different than resting cells. In mesenchymal cells, such as chondrocytes, such tests can be used to identify the differentiation status of the cells. Using the method of the invention, the presence, absence or mutant expression of ILA can be identified in a cell and the appropriate course of treatment can be employed (e.g., sense or antisense gene therapy).

The ILA-specific antibodies, ILA ligand, and ILA receptor inhibitors of the invention are suited for use, for example, in immunoassays in which they can

be utilized in liquid phase or bound to a solid phase carrier. In addition, the monoclonal antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize monoclonal antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the monoclonal antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The monoclonal antibodies of the invention can be bound to many different carriers and used to detect the presence of ILA peptides. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such using routine experimentation.

For purposes of the invention, ILA may be detected by the monoclonal antibodies of the invention when present in biological fluids and tissues. Any sample containing a detectable amount of ILA can be used. A sample can be a liquid such as urine, saliva, cerebrospinal fluid, blood, serum and the like, or a solid or semi-solid such as tissues, feces, and the like, or, alternatively, a solid tissue such as those commonly used in histological diagnosis.

As used in this invention, the term "epitope" includes any determinant capable of specific interaction with the monoclonal antibodies of the invention. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

In using the monoclonal antibodies of the invention for the *in vivo* detection of antigen, the detectably labeled monoclonal antibody is given in a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to enable detection of the site having the ILA antigen for which the monoclonal antibodies are specific.

The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having ILA protein is detectable compared to the background. Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

As a rule, the dosage of detectably labeled monoclonal antibody for *in vivo* diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. The dosage of monoclonal antibody can vary from about 0.001 mg/m^2 to about 500 mg/m^2, preferably 0.1 mg/m^2 to about 200 mg/m^2, most preferably about 0.1 mg/m^2 to about 10 mg/m^2. Such dosages may vary, for example, depending on whether multiple injections are given, inflammatory burden, and other factors known to those of skill in the art.

For *In vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for *In vivo* diagnosis is that the half-life of the radioisotope be long enough so that it is still detectable at the time of maximum uptake by the target, but short enough so that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for *in vivo* imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may be readily detected by conventional gamma cameras.

For *In vivo* diagnosis, radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are ^{111}In, ^{97}Ru, ^{67}Ga, ^{68}Ga, ^{72}As, ^{89}Zr, and ^{201}Tl.

The monoclonal antibodies of the invention can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, and ^{56}Fe.

The monoclonal antibodies of the invention can be used to monitor the course of amelioration of ILA associated inflammatory host defense disorder. Thus, by measuring the increase or decrease in the number of cells expressing ILA or changes in the concentration of normal versus mutant ILA present in various body fluids and/or tissues, it would be possible to determine whether a particular therapeutic regiment aimed at ameliorating the disorder is effective.

The present invention also provides a method for treating a subject with a ILA associated host defense disorder. The ILA nucleotide sequence can be expressed in an altered manner as compared to expression in a normal cell; therefore it is possible to design appropriate therapeutic or diagnostic techniques directed to this sequence. Thus, where an inflammatory host defense disorder is associated with the over-expression of ILA, nucleic acid sequences that interfere with ILA expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific ILA mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme. In cases when a host defense disorder or abnormal cell phenotype is associated with the under expression of ILA or expression of a mutant ILA polypeptide, nucleic acid sequences encoding ILA (sense) could be administered to the subject with the disorder.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15

nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target ILA-producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal. Biochem.*, 172:289, 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J. Amer. Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

The present invention also provides gene therapy for the treatment of inflammatory host defense disorders which are mediated by ILA protein. Such therapy would achieve its therapeutic effect by introduction of the ILA antisense

polynucleotide, into cells of subjects having the host defense disorder. Delivery of antisense ILA polynucleotide can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. A construct containing the part of the gene encoding extracellular domain of the receptor would result in the production of soluble ILA receptor protein. This will bind and neutralize the biological consequences of ligand interaction with membrane-bound ILA. Disorders associated with under-expression of ILA could similarly be treated using gene therapy with sense nucleotide sequences.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a ILA sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific. Retroviral vectors can be made target specific by inserting, for example, a polynucleotide encoding an enzyme that determines the structure of a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target

specific delivery of the retroviral vector containing the ILA antisense polynucleotide.

Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence that enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines which have deletions of the packaging signal include but are not limited to Ψ2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes *gag, pol* and *env*, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for ILA antisense polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and

In vivo. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 um can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to brain cells in a biologically active form (Fraley, *et al., Trends Biochem. Sci*, 6:77, 1981). For instance, *in vivo* administration can be in a bolus or by gradual perfusion over time by means adapted for crossing the blood-brain barrier. For instance, the antibodies can be injected by epidural administration or intralumbar puncture using standard techniques well known to the medical profession.

In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be preser:. (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, *et al., Biotechniques*, 6:682, 1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on Ph, ionic strength, and the presence of divalent caticns.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid

moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes has been classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

In general, the compounds bound to the surface of the targeted delivery system will be ligands and receptors which will allow the targeted delivery system to find and "home in" on the desired cells. A ligand may be any compound of interest which will bind to another compound, such as a receptor.

Antibodies can be used to target liposomes to specific cell-surface ligands. For example, certain antigenic sites associated with ILA polypeptides in cells activated by proinflammatory agents may be exploited for the purpose of targeting ILA antibody-containing liposomes directly to the site of inflammation. Since the ILA gene product may be indiscriminate with respect to cell type in its action, a targeted delivery system offers a significant improvement over randomly administered non-specific liposomes. Preferably, the target tissue is articular cartilage tissue and the target cell is a primary chondrocyte.

A number of procedures can be used to covalently attach either polyclonal or monoclonal antibodies to a liposome bilayer. Antibody-targeted liposomes can include monoclonal or polyclonal antibodies or fragments thereof such as Fab, or $F(ab')_2$, as long as they bind efficiently to an antigenic epitope on the target cells. Liposomes may also be targeted to cells expressing receptors for hormones or other serum factors.

The antibodies and substantially purified ILA peptide of the present invention are ideally suited for the preparation of a kit. Such a kit may comprise a carrier means being compartmentalized to receive in close confinement therewith one or more container means such as vials, tubes and the like, each of the container means comprising the separate elements of the assay to be used.

The types of assays which can be incorporated in kit form are many, and include, for example, competitive and non-competitive assays. Typical examples of assays which can utilize the antibodies of the invention are radioimmunoassays (RIA), enzyme immunoassays (EIA), enzyme-linked

immunosorbent assays (ELISA), and immunometric, or sandwich immunoassays.

The term "immunometric assay" or "sandwich immunoassay", includes simultaneous sandwich, forward sandwich and reverse sandwich immunoassays. These terms are well understood by those skilled in the art. Those of skill will also appreciate that antibodies according to the present invention will be useful in other variations and forms of assays which are presently known or which may be developed in the future. These are intended to be included within the scope of the present invention.

In performing the assays it may be desirable to include certain "blockers" in the incubation medium (usually added with the labeled soluble antibody). The "blockers" are added to assure that non-specific proteins, proteases, or anti-heterophilic immunoglobulins to anti-HLA immunoglobulins present in the experimental sample do not cross-link or destroy the antibodies on the solid phase support, or the radiolabeled indicator antibody, to yield false positive or false negative results. The selection of "blockers" therefore may add substantially to the specificity of the assays described in the present invention.

It has been found that a number of nonrelevant (i.e., nonspecific) antibodies of the same class or subclass (isotype) as those used in the assays (e.g., IgG1, IgG2a, IgM, etc.) can be used as "blockers". The concentration of the "blockers" (normally 1-100 $\mu g/\mu l$) is important, in order to maintain the proper sensitivity yet inhibit any unwanted interference by mutually occurring cross reactive proteins in the specimen.

In addition to the polynucleotides of the invention, the monoclonal antibodies of the invention can also be used, alone or in combination with effector cells (Douillard, *et al., Hybridoma*, 5 Suppl:S139, 1986), for immunotherapy in an animal having an inflammatory host defense disorder which expresses ILA polypeptide with epitopes reactive with the monoclonal antibodies of the invention.

The proteinaceous drugs with which the monoclonal antibodies of the invention can be labeled include immunomodulators and other biological response modifiers. The term "biological response modifiers" encompasses substances which are involved in modifying the immune response in such manner as to inhibit formation of ILA receptors or to enhance the destruction of an ILA-associated cell for which the monoclonal antibodies of the invention are specific. Examples of immune response modifiers include such compounds as lymphokines. Lymphokines include tumor necrosis factor, the interleukins, lymphotoxin, macrophage activating factor, migration inhibition factor, colony stimulating factor, and interferon. Interferons with which the monoclonal antibodies of the invention can be labeled include alpha-interferon, beta-interferon and gamma-interferon and their subtypes.

It is believed that the native ligand for the ILA receptor is an as-yet-unidentified biological response modifier, and it is contemplated that disease states associated with expression of ILA may be ameliorated by *in vivo* administration to patients of a ligand, such as an antibody or other proteinaceous substance, that blocks the ILA receptor or binds to, and hence modifies the biological activity, of the native ligand for the ILA receptor.

It is also possible to utilize liposomes with the monoclonal antibodies of the invention in their membrane to specifically deliver the liposome to the area of the cell expressing ILA. These liposomes can be produced such that they contain, in addition to the monoclonal antibody, such immunotherapeutic agents as those described above which would then be released at the receptor site (Wolff, *et al., Biochemical et Biophysical Acta*, 802:259, 1984).

The monoclonal antibodies of the invention can be administered *in vivo* in a bolus, or by gradual perfusion over time, and by injection or in a dispersion system such as a the liposomes described above. The antibodies of the invention can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally, alone or in combination with effector cells or immune response modifiers. For instance, the antibodies can be administered parenterally by injection.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents and inert gases and the like.

The invention also relates to a method for preparing a medicament or pharmaceutical composition comprising the polynucleotides or the monoclonal antibodies of the invention, the medicament being used for therapy ILA-associated inflammatory host response disorders.

The following examples illustrate the manner in which the invention can be practiced. It is understood, however, that the examples are for the purpose of illustration and the invention is not to be regarded as limited to any of the specific materials or conditions therein.

EXAMPLE 1
Cells and Cell Cultures

Studies were performed using the following cells and cell cultures to determine whether mRNA for ILA could be detected and under what conditions it could be induced. Human peripheral blood mononuclear cells, monocytes, T and B lymphocytes were prepared as previously described (Villiger, *et al.*, *J. Immunol.*, 146:550-559, 1991). Chondrocytes (Villiger, *et al.*, *EMBO J.*, 11:135, 1992) and synoviocytes (Lotz, *et al.*, *J. Exp. Med.*, 167:1253, 1991) were isolated from tissues obtained from patients undergoing joint replacement or at autopsy from donors without known history of joint disease and prepared as described in Villiger, *et al.*, *supra*. Human thymocytes were isolated from tissue that was removed during cardiac surgery as previously described (Lotz, *et al.*, *supra*). HTLV-1 transformed T cell lines were obtained by co-culture of peripheral blood T cells with an irradiated HTLV-1-infected cell line (kindly provided by Dr. W. Wachsman, UCSD). B cells from the peripheral blood of normal donors were transformed with EBV by infection with EBV-containing supernatants from the B95-8 cell line obtained from ATCC. In addition, the following cell lines were obtained from ATCC: U373, glioblastoma; SD-N-MC, neuroblastoma; Weri, retinoblastoma; HS683, glioma; HEp-2 epithelial; HepG2 hepatoma; A549, lung carcinoma; U937, promonocyte; Raji, EBV-transformed B cells; HUT78, Jurkat and Molt4, T cell lines.

Obtaining the cDNA Sequence of ILA

Human peripheral blood mononuclear cells ("PBMC") were prepared and activated with PHA (phytohemagglutin) and/or PMA (phorbol 12-myristate 13-acetate) as previously described by Villiger, *et al.* (*J. Immunol., supra*). PCR analysis was performed on RNA samples from both resting and activated PBMC with primers corresponding to cell surface receptor genes. The sequences of the 5' and 3' primers used for amplification were AGG AGC AAG GAC CTG AGA CAT (SEQ. I.D. NO. 3) and AGC AGC AGG TCA CAG AG (SEQ. I.D. NO. 4), respectively. A 385 bp PCR product, which was not detected in unstimulated cells, but was induced by lymphocyte activation, was amplified on cDNA using 5' primer AGG AGC AAG GAC CTG AGA CAT (SEQ. I.D. NO. 3) and 3' primer AGC AGC AGG TCA CAG AG (SEQ. I.D. NO. 4). The PCR product was not detected in unstimulated cells but was induced by lymphocyte activation.

Using the PCR product as a probe, a 1419bp cDNA shown in Figure 1 (SEQ. I.D. NO. 1) having all the characteristics of a full length cDNA was isolated from a library of human T lymphocytes that had been transformed with human T-cell leukemia virus type 1 and activated with PHA/PMA. Using the method of Schall, *et al.* (*Cell*, 61:361, 1990), several separate library screenings and independent sequencing reactions were performed. Briefly, to obtain the complete cDNA sequence of this gene, the PCR product was used as a probe. Using an HTLV-1 transformed T cell line, a cDNA library was prepared with the SuperScript Plasmid System (BRL, Gaithersburg, MD). The library was plated, transferred to Biotrans A membranes (ICN, Costa Mesa, CA), and hybridized with gel-purified 400 bp DNA fragments. Positive clones were isolated and re-screened. Serial dilutions of the longest clone, a cDNA, were prepared as

described by S. Henikoff (*Gene*, 28:351-359). The cDNA sequence, shown in Figure 1, was obtained by automated sequencing (Applied Biosystems, Foster City, CA).

Sequence analysis of the selected cDNA revealed a single potential polyadenylation site (AATAAT) at position 1230-5, which is 45 bp upstream from a 20 nt poly A tail. In addition the ATG start codon at positions 1-3 is preceded by three in-frame translation stop codons (positions -96-4, -63-61, -54-52). Seven nucleotides (-5 to 4) surrounding the ATG codon are identical to the nine bp consensus sequence for initiation of translation (CCRCCAGG) as described by Kozak (*Cell*, 44:283-292, 1986). The translation stop codon TGA at position 766-8 terminates the only open reading frame of appropriate length.

EXAMPLE 2

Features of the Deduced ILA Amino Acid Sequence

The amino acid sequence of ILA (SEQ. I.D. NO. 2), deduced from the open reading frame of the cDNA, predicts a protein of 255 amino acids with a molecular mass of approximately 28 kD as shown in Figure 1. As shown in Figure 2, Kyte and Doolittle hydropathicity analysis (Kyte and Doolittle, *J. Mol. Biol.* 157:105-132, 1982) predicts a putative signal peptide (aa 1 to 17) and a 27 amino acid hydrophobic region as a potential transmembrane domain (aa 187 to 213) flanked by charged residues. These features suggest that ILA is an integral membrane protein. Following the 17 amino acid leader peptide is an extracellular domain of 169 amino acids, a transmembrane region of 27 amino acids and a intracellular domain of 42 amino acids.

Two potential N-glycosylation sites at amino acid positions 138 and 149 are based on the presence of the motif NSX/T where X is any amino acid except D or P). The serine at amino acid position 242 is a potential site for phosphorylation by protein kinase C. Two consecutive threonines at amino acid positions 234 and 235 are potential sites for phosphorylation by casein kinase II. In addition, amino acids 241-244 (CSCRFD) contains a potential binding site for the tyrosine kinase $p56^{lck}$.

EXAMPLE 3

Comparison of ILA to Other Members of the NGF/TNFR Family

The deduced amino acid sequences of ILA, CD40 (Stamenkovic, *et al., supra*), NGF receptor (Johnson, *et al., supra*), OX-40 (Mallett, *et al., supra*) TNF receptor II (Kohno, *et al., supra*) and the murine T-cell activation molecule 4-1BB, which is expressed by specific T-lymphocyte subsets (Kwon, *et al., supra*), were compared using the BestFit program of Genetics Computer Group. Consensus alignment of the three cysteine rich domains of ILA with those of other members of the NGF/TNF family and introduction of gaps aided maximum alignment.

As shown in Figure 3, the deduced amino acid sequences of ILA and 4-1BB display 73.6% similarity and 59.6% identity as determined by sequence comparison using NIH software. In addition, the extracellular domain of ILA contains 3 cysteine rich repeats, characteristic of members of the NGF/TNF superfamily at amino acids 48 to 86, 88 to 106, and 117 to 158. The threonines at amino acids 234 and 235 are similar to a conserved theonine residue also found in CD 40, CD 27, 4-1BB and Fas/APO-1 that has been

associated with signal transduction in CD40 (Inui, *et al.*, *supra*; (Knapp, *et al.*, eds.) pp. 93-95 Oxford University Press, Oxford, England, 1989).

EXAMPLE 4

Stimulation of PBMC with Mitogens

1. RNA preparation and Northern Blot Analysis

Various of the cell lines tested (described in Example 1 above) were analyzed for the presence of mRNA, indicating the production of ILA receptors in these cells.

RNA was isolated from unstimulated cells or from cultures that had been activated by mitogen stimulus for 11 hours with 1μg/ml of PHA or 1ng/ml of PMA (Sigma, St. Louis, MO). Total RNA of the above cell lines was isolated by the acid phenol method (Chomczynski, *et al.*, *Anal. Biochem.*, 162:156, 1987). Poly A+ RNA was obtained with PolyATract mRNA isolation system (Promega, Madison, WI). RNA was fractionated on 1% agarose-formaldehyde gels and transferred to Hybond N membranes (Amersham, Arlington Heights, IL).

RNA (up to 5 μg) was reverse-transcribed in a 20 μl volume containing 4 ml 5 x RT-buffer (Gibco-BRL, Gaithersburg, MD), 10 mM dithiothreitol (DTT) 500 mM dNTPs, 1 μl random hexanucleotides, 200 Units of MoMLV-RT (BRL), and 20 units of RNAsin (Promega Company, Madison, WI) for 30 - 120 minutes at 37 degrees C. Gel-purified DNA fragments were labeled with ^{32}P by random priming and used as probes. The 1.4 kb full length ILA cDNA was used as a probe.

2. Northern blot Analysis of Total RNA

Total RNA and poly A$^+$ RNA was analyzed for the presence of ILA transcripts by Northern blotting, using 15μg/lane for Total RNA and 1μg/ml for poly A$^+$ RNA. The filters were analyzed for the presence of ILA transcripts by Northern blotting. To document the amount of RNA loaded, the filters were subsequently analyzed for cyclophilin mRNA. RNA loads on the filters were determined by control hybridizations with a full-length cyclophilin cDNA probe (Hasel, *et al., Nucl. Acid Res.*, 18:4019, 1990). In some experiments (Figure 6) a glyceraldehyde 3-phosphate dehydrogenase (G3PDH) cDNA fragment was used for control hybridizations. A 190 bp fragment was amplified by reverse transcriptase-PCR using G3PDH primers sense: TGG TAT CGT GGA AGG ACT CAT GAC (SEQ. I.D. No. 5); antisense: ATG CCA GTG AGC TTC CCG TTC AGC (SEQ. I.D. NO. 6) and subcloned into pGEM3z (Promega Co., Madison, WI).

Autoradiographs were scanned on a Microtek MSF-300G scanner (Microtek International Inc., Taiwan) and bands were subsequently analyzed using NIH Image 1.44 software. Relative densities of hybridization signals were calculated in comparison to controls (unstimulated cells) for both ILA and cyclophilin hybridization signals. Calculations from ILA hybridizations were then corrected on the basis of those obtained in cyclophilin hybridizations in order to correct for variable RNA loads. The results of these studies are shown in Figure 4. By Northern blot analysis of poly A$^+$ RNA, three forms of ILA mRNA could be detected at 4.4 4.0 and 1.8 kb. But analysis of total RNA from stimulated PBMC (as shown in Figure 5) showed only the 4.4 kb form.

3. Quantitative PCR

To determine the time period during which mRNA was produced by activated cells and the amounts produced, PCR was conducted on activated cells over a time course at selected intervals. PCR was performed in the following example with 0.1 to 3 μl (depending on the primers) of the RT reaction product in 25 μl volume with 1 unit AmpliTaq (Perkin Elmer Cetus, Emeryville, CA), 140 mM dNTPs, 1.5 mM MgCl2, 10 mM Tris pH 8.3, 50 mM KCl and 10 pM of each primer. After a 5 minute denaturation step at 94 degrees C, the reaction proceeded in 35 cycles of 30 seconds at 94 degrees C, 30 seconds at 55 degrees C and 30 seconds at 74 degrees C, followed by 5 seconds at 72 degrees C.

Primers used for ILA PCR were:

ILA-START: GAG AAT TCC ATG GGA AAC AGC TGT TAC (SEQ. I.D. No. 7)

SKR6-SEN: AGG AGC AAG GAC CTG AGA CAT (SEQ. I.D. No. 3)

SKR6-AS: AGC AGC AGG TCA CAG AG (SEQ. I.D. No. 4)

ILA-BAC5': CAT TCC CGG GTC CTT GTA GTA AC (SEQ. I.D. No. 8)

ILA-BAC3': CGG TGA TCA TCC TGG CTC TCT CGC AGG GGC (SEQ. I.D. No. 9)

ILA-DEL1: TGC CTG CAT ATG TCA CAG (SEQ. I.D. No. 10)

ILA-DEL2: CAT ATG CAG GCA GAC CCT GGA CAA A (SEQ. I.D. No. 11)

PMBC were stimulated with 1μg/ml of PHA and 1ng/ml of PMA and RNA was isolated at time points of 4, 8, 18, 30 and 48 hours. Northern blots prepared as described above, except that each lane contained 11μg total RNA. Hybridization was performed with ^{32}P labeled ILA cDNA. These studies on kinetics showed that ILA mRNA was rapidly induced by stimulation with PHA and PMA within 1.5 hours, increased to maximal levels by 8 hours and declined

to near background levels by 48 hours (See Figure 5). However, ILA was not detectable in total or poly A$^+$ RNA from unstimulated cells from more than 20 normal donors.

EXAMPLE 5

Stimulation of PBMC with Anti-CD3 Antibodies

PBMC were also stimulated with anti-CD3 antibody (OKT3 1:5000). mRNA was isolated at the time points indicated above, and analyzed by RT-PCR using the primers ILA-BAC5' (SEQ. I.D. NO. 8) and ILA-BAC 3' (SEQ. I.D. NO. 9). To document equal amounts of template, the samples were also analyzed for G3PDH mRNA. Antibody to CD3 also induced ILA mRNA. As compared to stimulation with PHA and PMA, as shown in Figure 6, the effects of anti-CD3 had a slower onset, but a longer duration. ILA mRNA was first detected at 4 hours, reached the maximum at approximately 19 hours, and was still strongly expressed after 48 hours.

EXAMPLE 6

Preparation of ILA Deletion Clone by Quantitative PCR

An ILA deletion clone (ILA-DEL) was constructed by PCR-based mutagenesis which resulted in a deletion of 200 bp between nucleotide positions 340-359. The ILA cDNA from start to the HaeIII site at position 921 was subcloned into the EcoRI and SmaI site of pGEM7z (Promega) between the SP6 and T7 polymerase transcription start site. A fragment containing the 5' part of ILA was amplified using a SP6 primer and ILA-DEL1 (SEQ. I.D. NO. 10) ILA-DEL2 (SEQ. I.D. NO. 11) contained at its 5' end 22 nucleotides identical to the 3' end of the 5' ILA fragment. In a third PCR with the SP6 and T7 primers, the 5' and

3' ILA fragments served as templates for the generation of a ILA deletion fragment which lacks the 200 nucleotides located between ILA-DEL1 and ILA-DEL2. This fragment was subcloned into the EcoRI and HindIII sites of pGEM4z.

EXAMPLE 7

Stability of ILA mRNA

Actinomycin D (actD), a transcriptional inhibitor, was used to determine ILA mRNA stability in PHA/PMA stimulated and actinomycin D (actD) treated PBMC. Cells were stimulated with 1 µg/ml of PHA and 1 ng/ml of PMA for 4 hours before 5 µg/ml of actD (Sigma, St. Louis) was added. Cells were collected at different time points for analysis by RT-PCR as described above for expression of ILA mRNA, using as primers ILA-START (SEQ. I.D. NO. 7) and ILA-BAC3' (SEQ. I.D. NO. 9).

For quantitative analysis of ILA mRNA levels a competitive PCR was performed by co-amplifying cDNA with THE ILA deletion clone (ILA-DEL), lacking a 200 bp region described in Example 6. Serial dilutions of this deletion clone were co-amplified with single stranded cDNA as described above. PCR products were separated on a 1.3% agarose gel and stained with ethidium bromide. Pictures were taken with a Land MP4 Polaroid camera on positive/negative Polaroid film #665. Negatives were scanned and evaluated as described for Northern blot analysis.

Figure 7A shows a titration of ILA-DEL against constant amounts of cDNA from the control and the 15 and 30 minute actD samples. Ethidium bromide stained gels showed the points of equal intensity between target and competitor

products shifting from 4-8x10^4 in the sample not treated with actD to 4x10^4 and 2x10^4 competitor molecules in the samples treated with actD for 15 and 30 minutes, respectively. To obtain a quantitative analysis of these results negatives of the photographs were scanned and the ratios of target to competitor products were plotted against the number of competitor molecules present in the reaction (Figure 7B). The equivalence point (1:1 ratio) of target to competitor products was reached at 3.2x10^4 competitor molecules for the control, at 2.5x10^4 competitor molecules for the 15 minute actD sample and at 1.5x10^4 competitor molecules for the 30 minute actD sample. These values represent a half life time of ILA and mRNA of 30 minutes.

EXAMPLE 8

Expression of ILA in Normal Cells

Normal human thymocytes prepared as in Example 1 were studied as a source of immature T lineage cells. They exhibited a similar time course of ILA mRNA induction as peripheral blood lymphocytes when stimulated as above described with PHA and PMA. Stimulation with PHA or PMA alone was sufficient for ILA mRNA induction. But ILA mRNA was not expressed in response to IL-1β (10 ng/ml), LIF (10 ng/ml), IFNγ (500 U/ml), LPS (1 μg/ml) or TNFα (100 u/ml) after 7 hours (not shown). The recombinant human cytokines IL-1β, IFNγ, LIF and TNFα were purchased from R&D Systems (Minneapolis, MN). Endotoxin content was less than 0.1 ng per mg of cytokine protein. Analysis of T cell lines showed ILA expression in HTLV-1 transformed T cells and in Jurkat cells after stimulation with PHA and PMA, but ILA could not be detected in the T-cell lines Hut78 and MOLT4, even after stimulation with PHA and PMA.

EXAMPLE 9

ILA can be induced in normal B lymphocytes and is constitutively expressed in transformed B cells

In B lymphocytes purified from human peripheral blood as described in Example 1, ILA was detected by RT-PCR in cells that had been activated with anti-μ (12.5 μg/ml) or PMA (10 ng/ml) for 6 hours by analysis for ILA mRNA using RT-PCR with primers SKR6-SEN (SEQ I.D. NO 3) and -SKR 6-AS (SEQ I.D. NO 4). A series of B cell lines showed the presence of ILA mRNA in unstimulated Raji cells, an EBV-transformed B cell line, as well as in B cells from normal donors that had been transformed by EBV *in vitro*. Stimulation with PMA (10 ng/ml) had no detectable effect on the constitutive ILA mRNA expression in EBV transformed B cells. The results of these studies are shown in Figure 8.

EXAMPLE 10

ILA expression in mononuclear phagocytes

In human blood monocytes ILA was induced by stimulation with IL-1β or PMA (0.1 ng/ml) for 4 hours. RNA was isolated after 4 hours and analyzed for ILA mRNA by Northern blotting as shown in Figure 9A. Since the load of RNA differed considerably in this experiment a rehybridization of the blot with G3PDH cDNA is shown in Figure 9B. Lane 1: control; Lane 2: PMA (100 fg/ml); Lane 3: PMA (1 ng/ml); Lane 4: PMA (10 ng/ml); Lane 5: PMA (50 ng/ml); Lane 6: IL-1β 10 ng/ml. Figure 9C shows the results after densitometry and normalization of the ILA signals on the basis of G3PDH signals.

As shown in Figure 9, the effects of PMA were dose dependent with induction occurring at 0.1 ng/mL. The same three mRNA isoforms of 4.4, 4.0 and 1.8 kb as in lymphocytes were also detected in monocytes. In addition to PMA and IL-1β, ILA was also induced in monocytes by stimulation with lipopolysaccharide (LPS) (not shown). ILA was also found in the human premonocytic cell line U937 and *in vitro* derived macrophages after induction with IL-1β (10 ng/ml) and PMA (10 ng/ml) (not shown).

EXAMPLE 11

Tissue distribution and regulation of ILA expression in non-lymphoid cells

To determine whether ILA expression is restricted to the immune system, primary cells and cell lines from other tissues were examined. ILA and mRNA was not detectable by Northern blotting or PCR in unstimulated human articular chondrocytes, rheumatoid synoviocyte, or in several human tumor cell lines including the hepatoma HepG2. However, following activation with IL-1β, ILA mRNA was detected in several nonlymphoid cell types. HEp2 (epithelioid), HepG2 (hepatoma) cells or human articular chondrocytes were stimulated with IL-1β (10 ng/ml) for 6 hours. The presence of ILA mRNA was determined by PCR, using the primers ILA-BACS' and -3' (SEQ I.D. NOS. 8 and NO. 9, respectively). To document equal amount of template, the different samples were also analyzed for G3PDH and mRNA using P: PhiX 174, Hae III markers.

Figure 10 shows representative results for epithelial and hepatoma cells, and for human articular chondrocytes where this mRNA was not present in unstimulated cells but increased strongly in response to IL-1β (10 ng/ml). Among the non-lymphoid cell types tested, the lung carcinoma A549 expressed

ILA mRNA constitutively, but ILA was not inducible in the brain-derived cell lines Weri, HS683, U373 and SK-N-MC in response to IL-1β (10 ng/ml), IFNγ (500 U/ml) or by PMA (5 ng/ml) plus the calcium ionophore A23187 (1 nM) (Figure 11). These findings demonstrate that ILA is inducible by the proinflammatory cytokine IL-1β in diverse cell types and thus has a much wider tissue distribution than described for 4-1BB (Pollok, *et al.*, *J. Immunol.*, 150:771, 1993; Kwon, *et al.*, *Cell. Immunol.*, 121:414, 1989).

In another study ILA was induced in primary chondrocytes by the second messenger agonists PMA, cAMP, the calcium ionophore A23187, and by cyclohexemide (CHX). Its induction by CHX suggests that ILA accumulation in primary chondrocyte is suppressed by a labile protein that is constitutively being synthesized.

The foregoing description of the invention is exemplary for purposes of illustration and explanation. It should be understood that various modifications can be made without departing from the spirit and scope of the invention. Accordingly, the following claims are intended to be interpreted to embrace all such modifications.

SUMMARY OF SEQUENCES

Sequence I.D. No. 1 is a nucleotide sequence encoding ILA.

Sequence I.D. No. 2 is an amino acid sequence for ILA.

Sequence I.D. No. 3 is a nucleotide sequence for a 5' primer (SKR6-SEN) for ILA RNA.

Sequence I.D. No. 4 is a nucleotide sequence for a 3' primer (SKR6-AS) for ILA RNA.

Sequence I.D. No. 5 is a nucleotide sequence for a sense primer for ILA RNA.

Sequence I.D. No. 6 is a nucleotide sequence for an antisense primer for ILA RNA.

Sequence I.D. No. 7 is a nucleotide sequence for primer (ILA-START) for ILA RNA.

Sequence I.D. No. 8 is a nucleotide sequence for primer (ILA-BAC5') for ILA RNA.

Sequence I.D. No. 9 is a nucleotide sequence for primer (ILA-BAC3') for ILA RNA.

Sequence I.D. No. 10 is a nucleotide sequence for primer (ILA-DEL1) for ILA cDNA.

Sequence I.D. No. 11 is a nucleotide sequence for primer (ILA-DEL2) for ILA cDNA.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Lotz, Martin
Schwarz, Herbert

(ii) TITLE OF INVENTION: A RECEPTOR INDUCED BY LYMPHOCYTE ACTIVATION IN INFLAMMATORY RESPONSE

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: Los Angeles
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 90067

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE: 27-SEP-1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Boscich, June M.
(B) REGISTRATION NUMBER: 31,238
(C) REFERENCE/DOCKET NUMBER: PD-2965

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (619) 455-5100
(B) TELEFAX: (619) 455-5110

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1439 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: ILA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 140..904

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ACCTGTGCCA GATTTCATC ATG GGA AAC AGC TGT TAC AAC ATA GTA GCC ACT       172
                     Met Gly Asn Ser Cys Tyr Asn Ile Val Ala Thr
                       1               5                  10

CTG TTG CTG GTC CTC AAC TTT GAG AGC ACA AGA TCA TTG CAG GAT CCT       220
Leu Leu Leu Val Leu Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro
            15                  20                  25

TGT AGT AAC TGC CCA GCT GGT ACA TTC TGT GAT AAT AAC AGG AAT CAG       268
Cys Ser Asn Cys Pro Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln
        30                  35                  40

ATT TGC AGT CCC TGT CCT CCA AAT AGT TTC TCC AGC GCA GGT GGA CAA       316
Ile Cys Ser Pro Cys Pro Pro Asn Ser Phe Ser Ser Ala Gly Gly Gln
    45                  50                  55
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AGG ACC TGT GAC ATA TGC AGG CAG TGT AAA GGT GTT TTC AGG ACC AGG                364
Arg Thr Cys Asp Ile Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg
 60                  65                  70                  75

AAG GAG TGT TCC TCC ACC AGC AAT GCA GAG TGT GAC TGC ACT CCA GGG                412
Lys Glu Cys Ser Ser Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro Gly
                 80                  85                  90

TTT CAC TGC CTG GGG GCA GGA TGC AGC ATG TGT GAA CAG GAT TGT AGA                460
Phe His Cys Leu Gly Ala Gly Cys Ser Met Cys Glu Gln Asp Cys Arg
            95                 100                 105

CAA GGT CAA GAA CTG ACA AAA AAA GGT TGT AAA GAC TGT TGC TTT GGG                508
Gln Gly Gln Glu Leu Thr Lys Lys Gly Cys Lys Asp Cys Cys Phe Gly
       110                 115                 120

ACA TTT AAC GAT CAG AAA CGT GGC ATC TGT CGA CCC TGG ACA AAC TGT                556
Thr Phe Asn Asp Gln Lys Arg Gly Ile Cys Arg Pro Trp Thr Asn Cys
   125                 130                 135

TCT TTG GAT GGA AAG TCT GTG CTT GTG AAT GGG ACG AAG GAG ACG GAC                604
Ser Leu Asp Gly Lys Ser Val Leu Val Asn Gly Thr Lys Glu Arg Asp
140                 145                 150                 155

GTG GTC TGT GGA CCA TCT CCA GCC GAC CTC TCT CCG GGA GCA TCC TCT                652
Val Val Cys Gly Pro Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser
                160                 165                 170

GTG ACG CCG CCT GCC CCT GCG AGA GAG CCA GGA CAC TCT CCG CAG ATC                700
Val Thr Pro Pro Ala Pro Ala Arg Glu Pro Gly His Ser Pro Gln Ile
            175                 180                 185

ATC TCC TTC TTT CTT GCG CTG ACG TCG ACT GCG TTG CTC TTC CTG CTG                748
Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu
       190                 195                 200

TTC TTC CTC ACG CTC CGT TTC TCT GTT GTT AAA CGG CGC AGA AAG AAA                796
Phe Phe Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys
   205                 210                 215
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CTC CTG TAT ATA TTC AAA CAA CCA TTT ATG AGA CCA GTA CAA ACT ACT      844
Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr
220                 225                 230                 235

CAA GAG GAA GAT GGC TGT AGC TGC CGA TTT CCA GAA GAA GAA GAA GGA      892
Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly
                240                 245                 250

GGA TGT GAA CTG TGAAATGGAA GTCAATAGGG CTGTTGGGAC TTTCTTGAAA          944
Gly Cys Glu Leu
            255

AGAAGCAAGG AAATATGAGT CATCCGCTAT CACAGCTTTC AAAAGCAAGA ACACCATCCT    1004

ACATAATACC CAGGATTCCC CCAACACACG TTCTTTTCTA AATGCCAATG AGTTGGCCTT    1064

TAAAAATCCA CCACTTTTTT TTTTTTTTTG CACAGCGTCT CACTCTGTCA CCCAGGCTGG    1124

AGTGCAGTGG CACCACCATG GCTCTCTGCA GCCTTGACCT CTGGGAGGTC AAGTGATCCT    1184

CCTGCCTCAG TCTCCTGAGT AGCTGGAACT ACAAGGAAGC GCCACCACAC CTGACTAACT    1244

TTTTTGTTTT TTGTTGGTAA AGATGGCATT TCGCCATGTT GTACAGGCTG GTCTCAAACT    1304

CCTAGGTTCA CTTTGGCCTC CCAAAGTGCT GGGATTACAG ACATGAAGTG CCAGGCCCGG    1364

CCAAAATAAT GCACCACTTT TAACAGAACA CACAGATGAG CACAGAGGTG GTGATAAAAA    1424

AAAAAAAAAA AAAAA                                                     1439
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 255 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gly Asn Ser Cys Tyr Asn Ile Val Ala Thr Leu Leu Leu Val Leu
1 5 10 15

Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn Cys Pro
20 25 30

Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln Ile Cys Ser Pro Cys
35 40 45

Pro Pro Asn Ser Phe Ser Ser Ala Gly Gly Gln Arg Thr Cys Asp Ile
50 55 60

Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg Lys Glu Cys Ser Ser
65 70 75 80

Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro Gly Phe His Cys Leu Gly
85 90 95

Ala Gly Cys Ser Met Cys Glu Gln Asp Cys Arg Gln Gly Gln Glu Leu
100 105 110

Thr Lys Lys Gly Cys Lys Asp Cys Cys Phe Gly Thr Phe Asn Asp Gln
115 120 125

Lys Arg Gly Ile Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Lys
130 135 140

Ser Val Leu Val Asn Gly Thr Lys Glu Arg Asp Val Val Cys Gly Pro
145 150 155 160

Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala
165 170 175

Pro Ala Arg Glu Pro Gly His Ser Pro Gln Ile Ile Ser Phe Phe Leu
180 185 190

Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu
195 200 205

Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe
210 215 220

Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
225 230 235 240

Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
245 250 255

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: SKR6-SEM

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION: 1..21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGGAGCAAGG ACGTGAGACA T 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: SKR6-AS

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION: 1..17

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ACCAGCAGGT CACAGAG 17

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: G3PDH PCR

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TGGTATCGTG GAAGGACTCA TGAC 24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vii) IMMEDIATE SOURCE:
(B) CLONE: GAPDH PCR

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: ILA-START

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAGAATTCCA TCGCAAACAG CTGTTAC 27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: ILA-BAC5.

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION: 1..23

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CATTCCCCGG TCCTTGTAGT AAC 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: ILA-BAC)'

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCGTCATCAT CCTGGCTCTC TCGCAGGGGC 30

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: ILA-DEL1

(ix) FEATURE:
(A) NAME/KEY. misc_RNA
(B) LOCATION: 1..18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGCCTGCATA TGTCACAG 18

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: ILA-DEL2

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION: 1..25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CATATCCAGG CAGACCCTCG ACAAA

CLAIMS

1. An isolated polypeptide comprising all or a portion of ILA.

2. An isolated polynucleotide which encodes the polypeptide of claim 1.

3. The polynucleotide of claim 2, wherein the polynucleotide is DNA.

4. The polynucleotide of claim 2, wherein the polynucleotide is RNA.

5. A host cell containing the polynucleotide of claim 2.

6. A recombinant expression vector containing the polynucleotide of claim 2.

7. The vector of claim 6, wherein the polynucleotide is an antisense sequence.

8. The vector of claim 6, which is a virus.

9. The vector of claim 8, wherein the virus is an RNA virus.

10. The vector of claim 9, wherein the RNA virus is a retrovirus.

11. The vector of claim 6, wherein the vector is a colloidal dispersion system.

12. The vector of claim 11, wherein the colloidal dispersion system is a liposome.

13. The vector of claim 12, wherein the liposome is essentially target specific.

14. The vector of claim 13, wherein the liposome is anatomically targeted.

15. The vector of claim 13, wherein the liposome is mechanistically targeted.

16. The vector of claim 15, wherein the mechanistic targeting is passive.

17. The vector of claim 15, wherein the mechanistic targeting is active.

18. The vector of claim 17, wherein the liposome is actively targeted by coupling with a moiety selected from the group consisting of a sugar, a glycolipid, and a protein.

19. The vector of claim 18, wherein the protein moiety is an antibody, or fragment thereof.

20. The vector of claim 6, wherein the vector is a plasmid.

21. Antibodies which are immunoreactive with the polypeptide of claim 1, or fragments thereof.

22. The antibodies of claim 21, wherein the antibodies are polyclonal.

23. The antibodies of claim 21, wherein the antibodies are monoclonal.

24. A method for identifying a cell expressing ILA comprising contacting a cell component with a reagent which binds to the component.

25. The method of claim 24, wherein the component is nucleic acid.

26. The method of claim 24, wherein the component is protein.

27. The method of claim 25, wherein the nucleic acid is DNA.

28. The method of claim 25, wherein the nucleic acid is RNA.

29. The method of claim 24, wherein the reagent is a probe.

30. The method of claim 29, wherein the probe is nucleic acid.

31. The method of claim 29, wherein the probe is an antibody.

32. The method of claim 31, wherein the antibody is polyclonal.

33. The method of claim 31, wherein the antibody is monoclonal.

34. The method of claim 24, wherein the cell is a lymphoid cell.

35. The method of claim 24, wherein the cell is a mesenchymal cell.

36. The method of claim 29, wherein the probe is detectably labeled.

37. A method for identifying host defense inflammatory response in body tissue comprising contacting a body tissue sample with an ILA agent.

38. The method of claim 37, wherein the tissue is articular cartilage and the host defense response is rheumatoid arthritis.

39. The method of claim 37, wherein the agent is a probe.

40. The method of claim 37, wherein the probe is an antibody.

41. The method of claim 40, wherein the antibody is polyclonal.

42. The method of claim 40, wherein the antibody is monoclonal.

43. The method of claim 40 or 41 wherein the antibody is detectably labeled.

44. The method of claim 43, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

45. A method of mediating an inflammatory host defense response disorder associated with ILA comprising administering to a subject with the disorder, a therapeutically effective amount of a reagent which modulates ILA activity.

46. The method of claim 45, wherein the reagent is an antisense polynucleotide sequence.

47. The method of claim 45, wherein the reagent is an antibody.

48. The method of claim 45, wherein the antibody is monoclonal.

49. The method of claim 45, wherein the disorder is rheumatoid arthritis.

50. The method of claim 45, wherein the reagent is a sense polynucleotide sequence.

51. An isolated polynucleotide sequence which comprises 5' and 3' untranslated nucleotide sequences associated with the nucleotide sequence which encodes ILA.

52. The polynucleotide of claim 2, wherein the polynucleotide is Sequence I.D. No 1.

53. An isolated polynucleotide sequence which comprises nucleotide sequence encoding ILA polypeptide, or a fragment thereof.

54. An isolated polypeptide wherein the polypeptide is encoded by the polynucleotide of Sequence ID. No. 1.

55. The polypeptide of claim 54, wherein the polypeptide is Sequence I.D. No. 2.

56. The polypeptide of claim 55 wherein the polypeptide is a fragment of the extra cellular domain.

57. A method of ameliorating an ILA-mediated pathology in an animal comprising:
administering to the animal a therapeutically effective amount of an ILA agent.

58. The method of claim 57, wherein the ILA agent inhibits adhesion between the ILA receptor and a ligand therefor.

59. A pharmaceutical composition comprising ILA-mediated pathology ameliorating amounts of an ILA agent together with a pharmaceutically inert carrier.

60. A method of detecting ILA-mediated pathology which comprises contacting a source suspected of containing ILA receptor with a diagnostically effective amount of detectably labeled ILA agent and determining if the ILA agent binds with the ILA receptor.

61. The method of claim 60, wherein the ILA agent inhibits adhesion between the ILA receptor and an ILA ligand.

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Patent Agents

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-139                                                                  CCACGCGTCCCAG
-126 ACCAAGGAGTGGAAAGTTCTCCGGCCAGCGCTGAGATCTCAAGAGTCACATTTGTCACACCAGC
 -63 TAATTTCATTAAAATTCTCTTGGAATCAGCTTTGCTAGTATCATACCTGTGCCAGATTTCATC

   1 ATG GGA AAC AGC TGT TAC AAC ATA GTA GCC ACT CTG TTG CTG GTC CTC
   1 Met Gly Asn Ser Cys Tyr Asn Ile Val Ala Thr Leu Leu Leu Val Leu

  49 AAC TTT GAG AGG ACA AGA TCA TTG CAG GAT CCT TGT AGT AAC TGC CCA
  17 Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn Cys Pro

  97 GCT GGT ACA TTC TGT GAT AAT AAC AGG AAT CAG ATT TGC AGT CCC TGT
  33 Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln Ile Cys Ser Pro Cys

 145 CCT CCA AAT AGT TTC TCC AGC GCA GGT GGA CAA AGG ACC TGT GAC ATA
  49 Pro Pro Asn Ser Phe Ser Ser Ala Gly Gly Gln Arg Thr Cys Asp Ile

 193 TGC AGG CAG TGT AAA GGT GTT TTC AGG ACC AGG AAG GAG TGT TCC TCC
  65 Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg Lys Glu Cys Ser Ser

 241 ACC AGC AAT GCA GAG TGT GAC TGC ACT CCA GGG TTT CAC TGC CTG GGG
  81 Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro Gly Phe His Cys Leu Gly

 289 GCA GGA TGC AGC ATG TGT GAA CAG GAT TGT AGA GAA GGT CAA GAA CTG
  97 Ala Gly Cys Ser Met Cys Glu Gln Asp Cys Arg Glu Gly Gln Glu Leu

 337 ACA AAA AAA GGT TGT AAA GAC TGT TGC TTT GGG ACA TTT AAC GAT CAG
 113 Thr Lys Lys Gly Cys Lys Asp Cys Cys Phe Gly Thr Phe Asn Asp Gln

 385 AAA CGT GGC ATC TGT GCA CCC TGG ACA AAC AGT TCT TTG GAT GGA AAG
 129 Lys Arg Gly Ile Cys Ala Pro Trp Thr Asn Cys Ser Leu Asp Gly Lys

 433 TCT GTG CTT GTG AAT GGC ACC AAG GAG AGG GAC GTG GTC TGT GGA CCA
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 481 TCT CCA GCC GAC CTC TCT GGG GCA GCA TCC TCT GTG ACC CCG CCT GCC
 161 Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala

 529 CCT TCC AGA GAG CCA GGA CAC TCT CCC CAG ATC ATC TCC TTC TTT CTT
 177 Pro Ala Arg Glu Pro Gly His Ser Pro Gln Ile Ile Ser Phe Phe Leu

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 625 CGT TTC TCT GTT GTT AAA CGG GGC AGA AAG AAA CTC CTG TAT ATA TTC
 209 Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe

 673 AAA CAA CCA TTT ATG AGA CCA GTA CAA ACT ACT CAA GAG GAA GAT GGC
 225 Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
                                                  CKII
 721 TGT AGC TGC CGA TTT CCA GAA GAA GAA GAA GGA GGA TGT GAA CTG TGA
 241 Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu ---
       PKC

 769 AATGGAAGTCAATAGGGCTGTTGGGACTTTCTTGAAAGAAGCAAGGAAATATCAGTCATCCGC
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1153 CTGGTCTCAAACTCCTAGGTTCAGTTTGCCTCCCAAAGTGCTGGGATTACAGACATGAACTGC
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1281 T(A)n
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FIGURE 1

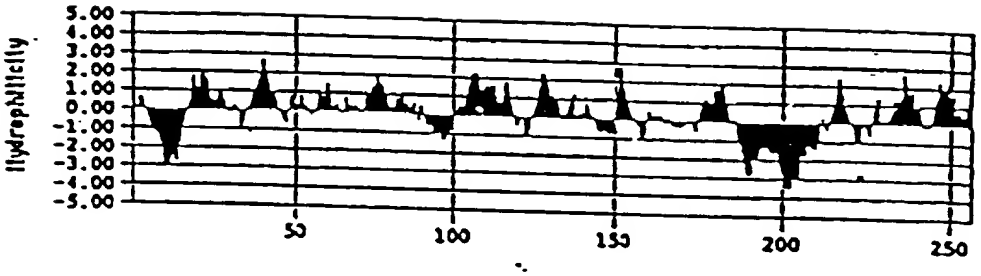

FIGURE 2

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FIGURE 3

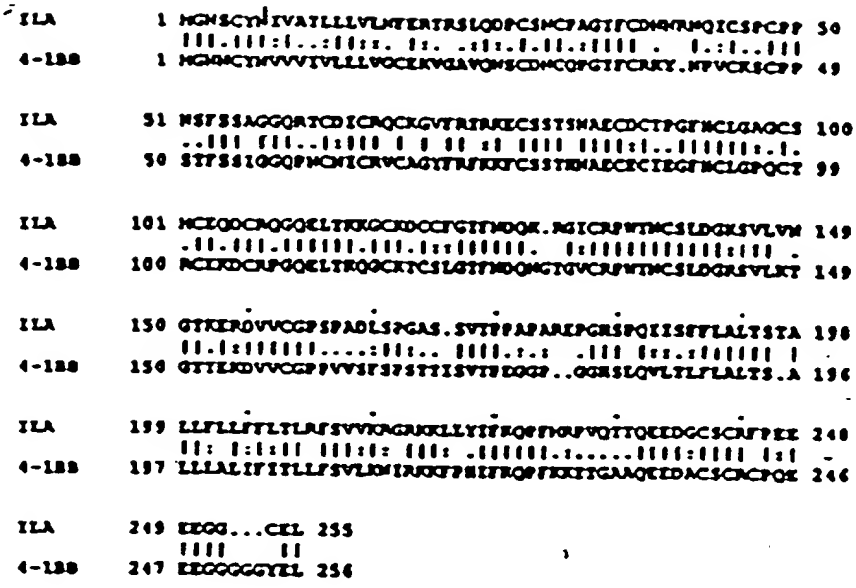

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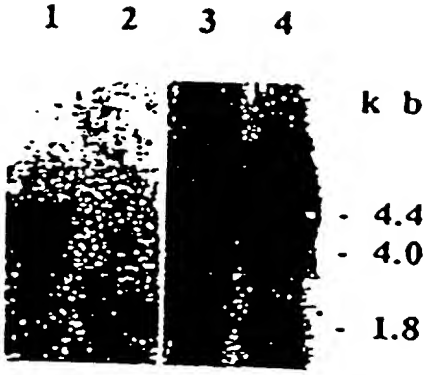
FIGURE 4

A



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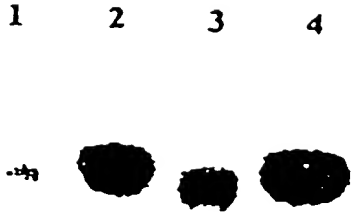

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FIGURE 5

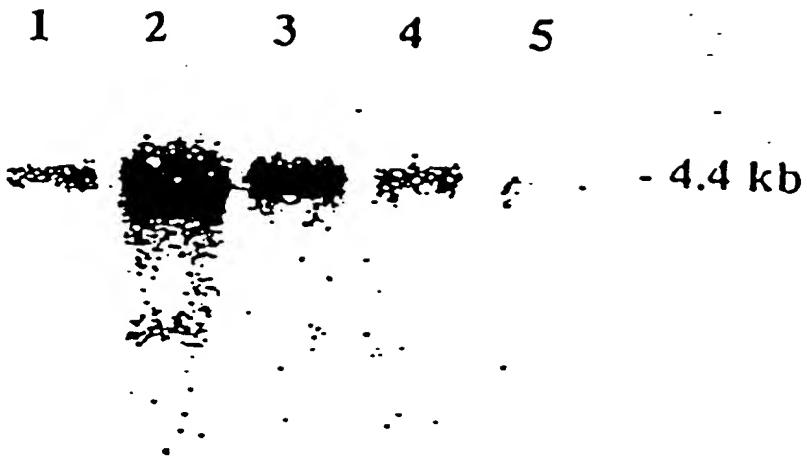

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FIGURE 6